Detection of antineutrophil cytoplasmic antibody in a patient with L-tryptophan induced eosinophilia-myalgia syndrome

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Abstract
The Center for Disease Control has received numerous reports of an eosinophilia-myalgia syndrome related to products containing L-tryptophan. The case is reported of eosinophilia-myalgia syndrome and polyneuropathy associated with myeloperoxidase specific antineutrophil cytoplasmic antibody.

Case report
A 61 year old woman presented in November 1989 with diffuse myalgias, fatigue, and a diffuse, pruritic, maculopapular rash, followed by shortness of breath. There was no history of ingestion of raw meats, recent travel, or contact with ill persons. Her only drug at the time of onset of symptoms was L-tryptophan, 1000 mg (unknown manufacturer) at bedtime for insomnia, which she had been taking for four years. There were no known allergies.

On initial examination she was afebrile. There was diffuse muscular tenderness without weakness. Bibasilar rales were noted. Electrocardiogram monitoring showed episodes of paroxysmal atrial tachycardia. Chest radiography showed mild congestive heart failure.

The leucocyte count was 13.0 x 10^9/l; haemoglobin 123 g/l; packed cell volume 0.46; and platelet count 147 x 10^9/l. Absolute eosinophil count was 0.214 x 10^9 cells/l (normal 0-0.474 x 10^9 cells/l). Total protein was 56 g/l; albumin 15 g/l; phosphorus 0.936 mmol/l; serum sodium 129 mmol/l, alkaline phosphatase 2.72 µkat/l, and lactate dehydrogenase 5.45 µkat/l. Renal function and results of urine analysis were normal. Arterial blood gases obtained on room air showed pH 7.42, PaO2 37 mmHg, and PaCO2 49 mmHg. Chest radiography showed small bilateral pleural effusions without evidence of congestive heart failure. Antinuclear antibody was positive (1/5120), whereas antihistone, double stranded DNA, SS-A, SS-B, anticentromere, Scl-70, anti-RNP, and cryoglobulins were all negative. Antineutrophil cytoplasmic antibody (ANCA) was positive (1/320, normal <1/40) with a fine, irregular granular cytoplasmic staining pattern (fig 1). Confirmatory enzyme immunoassays for antineutrophil cytoplasmic and antiprotease 3 showed ANCA specificity for antimyeloperoxidase. No antiprotease 3 activity was detected. Nerve conduction studies showed slowing in multiple segments with decreased peroneal continuous motor action potentials and absent sural responses; electromyography showed small, polyphasic, long duration units with early recruitment, compatible with a neuropathic process.

A right quadriceps muscle biopsy suggested severe neurogenic atrophy with secondary myopathic changes: frozen sections of skeletal muscle stained with haematoxylin/eosin showed that 90% of the fibres were atrophic. Many of these were angular in large groups. Many cells were basophilic and others overtly necrotic. There was an increase in endomysial connective tissue.

Figure 1 Antineutrophil cytoplasmic antibody staining demonstrated on ethanol fixed human neutrophils by indirect immunofluorescent microscopy.
tissue. Stains with periodic acid-Schiff reagent showed appropriate glycerogen. Congo red stains were negative for amyloid. On serial section one vessel had a number of inflammatory cells within the wall and in the perivascular connective tissue (fig 2). There was no evidence of thrombosis or necrosis of the vessel wall. Nasal mucosal biopsies did not show any evidence of granulomatous inflammation. Stomach and small bowel biopsies were negative for Ménétrier's disease.

Progressive worsening of respiratory muscle function necessitated the use of ventilatory support. Treatment was started with methylprednisolone (Solumedrol), 20 mg every eight hours, with improvement in respiratory inspiratory force and weaning from mechanical ventilatory support. The weakness in her upper and lower extremities increased, prompting empirical treatment with cyclophosphamide 2 mg/kg daily. This was temporarily withheld during an episode of bacterial pneumonia. With this regimen ventilatory support was not required and signs of gradual improvement in muscle strength were seen. After a complicated course she was finally discharged and continued to receive prednisone (35 mg daily) and cyclophosphamide (100 mg daily).

Discussion
The use of L-tryptophan has been implicated in a syndrome characterised by subacute onset of myalgia, fatigue, respiratory symptoms, including cough, dyspnoea, and hypoxaemia, skin rash (described as maculopapular, vesicular, or urticarial), and frank muscle weakness. Both marked peripheral eosinophilia and perivascular, eosinophilic inflammatory infiltrates are striking findings.

The incidence of antinuclear antibody production in eosinophilia-myalgia syndrome is unknown. Previously reported cases have not shown evidence of high titre antinuclear antibody, though Varga et al reported one patient with a titre of 1/2560 in association with diffuse fasciitis and L-tryptophan use. The development of polyneuropathy and progression of muscle weakness despite withdrawal of the offending agent and resolution of the eosinophilia has previously been noted in eosinophilia-myalgia syndrome. This suggests that the neuropsychiatric process can progress independently of circulating blood concentrations of L-tryptophan or eosinophilia.

Antineutrophil cytoplasmic antibody specificity has been the subject of two international workshops (Copenhagen, 1988; Leiden, 1989) and thus far, two major specificities have been identified: antineutrophilic proteinase 3 and antineutrophilic proteinase 4. These have been mutually exclusive in all reported studies.

The disease associations of ANCA have been the subject of a number of reports, but systemic vasculitides and glomerulonephritis characterised by segmental necrosis, epithelial crescent formation, and little or no immune deposits are most commonly identified with these autoantibodies. Several early reports closely linked ANCA to Wegener's granulomatosis.

The sensitivity and specificity of ANCA in active Wegener's granulomatosis has been reported to be as high as 93% and 97% respectively. In most cases of Wegener's granulomatosis antineutrophilic proteinase 3 ANCA specificity has been found, though in a smaller number of cases antineutrophilic proteinase 4 has been identified.

Positive ANCA have also been reported in idiopathic necrotising segmental crescentic glomerulonephitis, polyarteritis (both classical and microscopic variants), allergic granulomatosis of Churg-Strauss, and cystic fibrosis with vasculitis.

In patients with idiopathic necrotising segmental crescentic glomerulonephritis, both with and without associated systemic vasculitis, antineutrophilic proteinase 4 is the most commonly reported specificity; however, in some series antineutrophilic proteinase 3 has been found in a smaller number of patients with this diagnosis.

Even though a large number of other rheumatic diseases do not appear to be associated with ANCA, its presence in this patient with tryptophan associated eosinophilia-myalgia syndrome broadens the spectrum of possible associations.

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Corrected serum calcium and full blood count remained normal. A drug induced central nervous system toxicity syndrome was diagnosed by a psychiatrist, and thioridazine reduced the frequency and disturbing effect of the hallucinations. A brain computed tomographic scan was normal.

Studies of patients with Paget's disease treated with pamidronate have consistently shown a transient fall in serum calcium and phosphate, which are seldom of clinical significance and are associated with a decline in urinary calcium excretion and an increase in plasma parathyroid hormone levels. 6 Transient haematological changes and fevers have also been reported after both oral and IV pamidronate, possibly mediated through direct or indirect effects on mononuclear phagocytes, resulting in the activation of cytokines. 7 The mechanism underlying hallucinations in this patient is unknown but is considered unlikely to be due to alterations in serum calcium concentrations.

Adverse psychiatric reactions to bisphosphonates appear to be rare, although etidronate has previously been reported to cause confusion (Committee on Safety of Medicines, personal communication). It is recommended that the mental state of patients given high dose infusions of pamidronate for Paget's disease should be monitored closely after their treatment.

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Correction

Sir: We are writing to correct an inadvertent error in our manuscript ‘Detection of antineutrophil cytoplasmic antibody in a patient with L-tryptophan induced eosinophilia-myalgia syndrome’, which appeared in volume 50 of the Annals last year. 1 The caption of fig 1, on page 817, stated that the antineutrophil cytoplasmic antibody stain shown was demonstrated on ethanol fixed human neutrophils. This photomicrograph was actually of the antineutrophil cytoplasmic antibody indirect immunofluorescence on formalin-acetone fixed human neutrophils. This is of importance because the antineutrophil cytoplasmic antibody (ANCA) specificity documented by enzyme immunoassay was for myeloperoxidase, which typically produces a perinuclear/nuclear staining pattern on ethanol fixed neutrophils rather than the granular cytoplasmic staining which is depicted. This pattern on ethanol fixed neutrophils is associated with antiproteinase 3 specificity in about 85–90% of cases. An assay for antiproteinase 3 was negative in our patient, who also had a high titre of antinuclear antibody present at the time the ANCA was detected. Myeloperoxidase ANCA are difficult to detect on ethanol fixed neutrophils in the presence of antinuclear antibodies; therefore, we used the formalin-acetone fixation technique, which prevents the translocation of myeloperoxidase from the primary granules in the neutrophil cytoplasm to the nucleus when the nuclear membrane is lysed. When this technique is used, both types of ANCA demonstrate the staining pattern shown. In the absence of antinuclear antibodies ethanol fixed neutrophils are then used to rescreen the patient’s serum and if the pattern converts to a perinuclear/nuclear one, myeloperoxidase specificity is present in 90% of cases. When antinuclear antibodies obscure the ANCA pattern a secondary assay such as the enzyme linked immunoabsorbent assay (ELISA) we used must be employed to identify the specificity of the ANCA present.

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